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TITLE : COSMETIC MATERIAL

ABSTRACT : PROBLEM TO BE SOLVED: To obtain a cosmetic material useful for skin preparation and aging prevention by intending to maintain and promote healthy normal flora on the skin by including at least one or more materials selected from among specific metal ions, metal salts and metal materials.

SOLUTION: This cosmetic material contains a minute amount of one of manganese, zinc or their salts which have actions to promote growth of aerobically most dominant Staphylococcus epidermidis in a useful normal flora of the skin and enhance superoxide dismutase (SOD)-like active material excreted from the bacteria cells. The preferable concentration range of manganese ion is 0.1-100mM, especially 0.1-10mM and that of zinc ion is 0.01-5mM, especially 0.1-1mM.

* NOTICES *

CLAIMS

[Claim]
[Claim 1] Metal ion Metal salt Charge of makeup characterized by containing one or more of metal matter.
[Claim 2] The manganese with the operation which increases growth of aerobic maximum superior bacillus Staphylococcus ********** of a skin useful resident bacterial flora, and the operation which raises the super oxide dismutase (SOD) Mr. active substance in which the concerned biomass carries out production secretion which is a metal ion and zinc or those salts, for example, a manganese chloride, and a zinc oxide, or the charge of makeup characterized by carrying out minute amount inclusion of any one.
[Claim 3] The aforementioned manganese ion is a charge given in the claim 2 which is the concentration domain of 0.1-100mM, and contains the concentration domain of 0.1-10mM desirably of makeup.
[Claim 4] The aforementioned zinc ion is a charge given in the claim 2 which is the concentration domain of 0.01-5mM, and contains the concentration domain of 0.1-1mM desirably of makeup.

[Translation done.]

DETAILED DESCRIPTION

[Detailed description]
[0001]
[Field of the Invention] The use as the charge of makeup to which the charge of this makeup carries out the decomposition elimination of the active oxygen on the front face of the skin which is a powerful enemy at the skin by raising SOD Mr. active substance which aims at a maintenance and improvement of healthy skin normal bacterial flora congruence, and the skin indigenous bacteria secretes, and this invention is useful at the ready skin and skin aging prevention, and a skin medicine for external application is expected.
[0002]
[Prior art] Although it acts as the ** student of the detrimental active oxygen constantly for a living body in the organism which uses O2 for a metabolic system, SOD which carries out the catalyst of the disproportionation of the super oxide anion (O2-) which is a kind of active oxygen is a typical antioxidation enzyme, and it is said that the cell is protected from the oxygen trauma by this enzyme activity. Also in the health science of the skin, it was placed between the skin lipid oxidization by solar light ultraviolet rays by this active oxygen, acceleration of skin aging and the intervention to the trouble of the skin attracted attention, and the application to the charge of makeup or skin medicine for external application of SOD active substance has been proposed. (Publication number 1-96107)
[0003] However, the natural-product origin SOD refined from an animal or vegetation, and a microorganism or DNA recombination field origin SOD had left the problem also from [, such as a store stability in the inside of a tablet,] quality control from the manufacturing cost.
[0004] Recently, the bacillus grown in good mind by the skin indigenous bacteria which inhabits a skin front face is strong into oxygen, and since the ultraviolet rays of the direct sun are received, having high antioxidation ability [expose / in many cases / to active oxygen] is suggested. As for the maximum superior bacillus Staphylococcus **********, the proposal of cosmetics and a medicine for external application it blends them since the culture medium or its culture field debris shows antioxidation nature is actually also made by the typical aerotropism indigenous bacteria. (Publication number 6-271851) However, the problem remained in the instability as a tablet.
[0005]
[Object of the Invention] When SOD or the incubation anti-oxidant was directly blended with the charge of makeup like the above, the problem solving on quality control, such as the store stability of SOD activity at the time of use, remained in the smell or the color pan as a technical probrem. Thus, in spite of having been observed by the ready skin operation of SOD and the antioxidation matter many years, many points which should still solve the combination application to the charge of makeup are left behind. this invention is by blending the active substance which makes the SOD activity of the grown useful skin normal bacterial flora secrete efficiently rather than blending with the charge of direct makeup to offer the cheap and safe charge of skin antioxidation nature makeup which shows the aging prevention effect of the skin, and a ready skin operation so that the manifestation reservation of SOD or the SOD Mr. active substance can always be carried out stably on the skin.
[0006] Especially, the thing of the maximum dominance forms the stable resident skin bacterial flora about [1x104 //cm] by two by Staphylococcus ********** as an aerobe on the healthy Homo sapiens's skin. The fixing propagation of this Staphylococcus ********** is carried out on a skin front face by high antioxidation ability, and it secretes SOD Mr. active substance out of a biomass, eliminates O2- on the skin produced by ultraviolet rays etc. as a result, prevents the skin trauma, and is considered to have played the increase of the degree of health of the skin, the role which prevents infection of other bacteria. Actually, by the skin (rough skin) with trauma, such as atopic dermatitis, or the dry skin, the balance of a skin bacterial flora collapses and the detrimental bacillus belonging to Staphylococcus ********** or the Micrococcus is seen at a high rate. Since Staphylococcus ********** as a superior bacillus suppresses propagation of the above-mentioned detrimental bacillus, the slight skin trauma becomes possible [suppressing propagation of a disease germ and also expecting the improvement effect of the skin trauma] by offering the charge of makeup which can proliferate Staphylococcus ********** alternatively.
[0007]
[The means for solving a technical problem] By repeating a research zealously, in order to solve the above-mentioned problem, raising the SOD Mr. specific activity outside a biomass of Staphylococcus **********, and adding the manganese and zinc which are a metal ion also in an oligotrophication culture medium, this invention proliferated this bacillus for a short time, finds out that SOD Mr. active substance outside a biomass can be maintained for a long period of time, and came to carry out this invention.

[0008] That is, this invention is a charge of makeup which blends the matter which can be made to be able to increase Staphylococcus ********** which resides on the skin for a short period of time, and can raise SOD Mr. specific activity outside a biomass. Moreover, this charge of makeup is a safe charge of makeup with a high stability which attains the ready skin by raising normalization of a skin bacterial flora, and the activity of the biomass external-secretion SOD Mr. active substance. In order to more often explain this invention, an example is described below.
[0009] (1) As used for the following examples, the microorganism used in order to prove the efficiency nature of this invention is Staphylococcus ********** which is the typical skin normal bacterial flora which inhabits healthy people's skin, or is a Staphylococcus ********** bacillus as representation of a skin detrimental bacillus. However, as bacteria which exist in the skin, the bacteria used in order to carry out this invention have also included the thing of permeability, and can be used regardless of the strain of a gap, and strain.
[0010] (2) In order to investigate not only a usual eutrophy culture medium but metal-ion demand nature, the minimum growth culture medium was also used for the culture medium used in order to prove the efficiency nature of this invention. That is, in the minimum growth culture medium, what added the glucose and the yeast extract and added the calcium carbonate as an energy source for pH manufacture was made into the basal medium. Although bacterial metal demand nature was known, it added to the basal medium about the metal ion of some kinds including the manganese which serves as the active center of SOD beforehand, and the concentration to which growth prevention does not happen was examined. Therefore, the minimum growth culture medium used in this invention is defined as what added the metal of the minimum concentration which can grow a bacteria.
[0011] (3) The cultivation of the bacteria used in order to prove the efficiency nature of this invention cultivated bacteria with the rotary shaker (120rpm) under the 37-degree C good mind-condition using the above-mentioned strain and the culture medium.
[0012] (4) The sample-preparation method used in order to prove the efficiency nature of this invention is as follows. First, a cooling supercentrifuge (made in the Sakuma factory) separates into a biomass and a culture supernatant the bacillus which fully grew by the above-mentioned culture medium at 4 degrees C for 10000rpm, 10 minutes. Subsequently, it condensed as follows in SOD activity measurement of a culture supernatant. It freeze-dried by having added so that it might become the 75% of the last concentration about a cold acetone at a culture supernatant, having carried out the standing to -20 degree-C freezer, having carried out separation recovery of the formed precipitate with the cooling centrifugal separator, and having melted it in the little purified water, and the acetone-powder sample was obtained. What melted this powder field by the purified water was made into the biomass external-secretion nature component sample.
[0013] (5) The nitroblue-tetrazolium (NBT) method was used as measurement of SOD Mr. active substance used in order to prove the efficiency nature of this invention. This measuring method is comparatively simple of a number of measuring method, is high, and can be detected to ng order. [of photographic sensitivity] O2- is generated by the xanthine oxidase and the degree of coloring of the nitroblue tetrazolium by O2- is measured with an absorbance with a wavelength of 560nm. If O2- is dismutated by presence of SOD, coloring prevention of NBT will happen and it will be asked for SOD activity from this rate of prevention. When SOD Mr. active substance secreted out of a biomass using this technique combined with acetone concentration processing of a culture supernatant sample and used, measurement became possible by high photographic sensitivity.
[0014] Hereafter, an example explains this invention still in detail. However, the domain of this invention is not restricted to the following examples.
[0015]
[Example]
Example 1 of an experiment The basal medium used for the experiment of a :series about the influence of the metal ion exerted on growth of Staphylococcus ********** is a yeast extract to the Davis culture medium (0.5g / disodium hydrogenphosphate 12 hydrate, 8g; potassium-dihydrogenphosphate, 2g; sodium-citrate, 0.5g; magnesium sulfate 7 hydrate, 0.1g; ammonium sulfate, 1g; glucose, and distilled water 1l. (pH7)). What added 0.1% and the calcium carbonate 1% It considered as 1x105 inoculation bacilli/ml, and incubation was performed by the 37-degree C thermostat. The metals used for the experiment are a copper sulfate, a zinc sulfate, a manganese chloride, a zinc oxide, and a ferrous sulfate, and concentration is each. It was referred to as 100microM, 1mM, and 10mM, and added before incubation start. Culture medium was applied to the standard agar plate after incubation of 24 hours, and the number of micro organisms was measured by constant law. The result is shown in Table 1.
[Table 1]
表1　スタフィロコッカス・エピデルミデスの発育に及ぼす金属イオンの影響

添加金属イオン	添加濃度（mM）		
	10	1	0.1
硫酸銅	−−	−	+/−
硫酸亜鉛	−	+	+/−
酸化亜鉛	−−	+/−	+
塩化マンガン	++	+++	++
硫酸第一鉄	−−	+	−

注)発育状態の表示法の意味；　++：著しく増加、+：増加、+/−：増減なし、−：低下、−−：死滅

[0016] As shown in Table 1, the growth facilitatory effect was looked at by the manganese chloride and the zinc sulfate, the zinc oxide, and the ferrous sulfate.
[0017] Example 2 of an experiment Since the experimental group which added the zinc sulfate and the manganese chloride

showed the value higher than a control group as a result of measuring SOD Mr. active substance of a Staphylococcus ********** culture supernatant in the example 1 of :experiment about the growth promotion and the prevention effect of a zinc oxide and a manganese chloride over the bacillus separated from the skin, these 2 **********s were observed. Using the zinc oxide widely used for cosmetics to the zinc sulfate at this time, the ferrous sulfate oxidized and was discolored, and since the skin might be affected, it excepted. Therefore, the proliferation profile of Staphylococcus ********** was examined using the culture medium which added the zinc oxide and the manganese chloride. Both metals concentration was set to 100microM, and inoculation **** was carried out in 1x106 pieces/ml. The result is shown in drawing 1 .

[0018] As shown in drawing 1 , in the mixture with a zinc oxide and a manganese chloride, increase remarkable in the growth ratio of Staphylococcus ********** accepted.

[0019] Moreover, the experiment with the same said of skin normal bacterial floras other than Staphylococcus ********** was conducted. The same experiment as the above-mentioned was conducted, using 671 stocks of Staphylococcus **********s IDD as a typical thing, and change of the number of bacilli was measured with the absorbance with a wavelength of 595nm. A result is shown in drawing 2 .

[0020] As shown in drawing 2 , by the zinc oxide with which Staphylococcus ********** which is a detrimental disease germ was added unlike useful indigenous-bacteria Staphylococcus **********, and the manganese chloride, special growth promotion did not accept and prevention did not accept, either.

[0021] Example 3 of an experiment The phenomenon which increases by influence [of the zinc oxide exerted on the SOD Mr. activity outside a biomass of Staphylococcus ********** and a manganese chloride]: next the zinc oxide of SOD Mr. specific activity outside a biomass found out in the example 1 of an experiment, and addition of a manganese chloride investigated as the following with regards to incubation time. Incubation was performed on the same conditions as the example 1 of an experiment, and SOD Mr. activity measured time progress later on. Per each experimental group, the number of samples was set to 3 and computed the average and standard deviation. Detailed explanation is further given about the experiment technique of the example 3 of an experiment below.

[0022] Sample-preparation Law: Adjustment of a sample performed incubation in the 100ml flask on the scale of 50ml culture medium, and it sampled 5ml at a time for every time. Centrifugal separation (10,000rpm, 4 degrees C, 10 minutes) of the culture medium was carried out immediately, and it was divided into the biomass and the supernatant liquid. The collected supernatant liquids are ice-cooled, it adds, stirring so that it may become 75% of final concentration, and is under ice-cooling, and a -20-degree C acetone is left for 2 hours. Centrifugal separation (10,000rpm, 4 degrees C, 10 minutes) was carried out again the back, and settlingss were collected. A settlings is melted in a little purified water, it freeze-dries by making it freeze at -80 degrees C, and an acetone is removed. Measurement of protein determination and SOD Mr. activity was performed, using what melted the obtained xeransis powder by the purified water as a sample.

[0023] Protein Assay: The Lowry method was used for a proteinic determination. Sample which contains 10-100microg for protein For a test tube, 2 ml addition of the alkali copper solution (what added 1ml of copper sulfates and the mixed liquor of 1ml of 2% Rochell salts to a sodium carbonate and 0.1N sodium-hydroxide 100ml 2% 1%) is carried out, 0.4 ml is stirred, and it is left for 10 minutes at a room temperature. After carrying out 0.2 ml addition stirring of the 1N phenol reagent immediately after that, it is left for 30 minutes at a room temperature. After 30 minutes 750 nm Colorimetry was carried out. The cow serum albumin (BSA, SIGMA company make) was made into the standard solution.

[0024] It is a sample to the measuring method:test tube of SOD Mr. active substance 50 mul is taken. 50microl addition of the XOD (xanthine oxidase) liquid (what was diluted so that XOD is melted in 0.5M ammonium sulfate and an EDTA-2Na 1 mM solution, and it might save at -20 degrees C, it might melt just before use and change of per minute of OD560nm might be set to 0.0185) is carried out, and it is put under 25 degree-C water bath for 10 minutes, without stirring. After stirring immediately A liquid (0.1 mM xanthin, a 0.025 mM nitroblue tetrazolium, 0.1 mM EDTA-2Na, 50 mM sodium-carbonate buffer solution (pH10.2)) warmed at 25 degrees C and setting it for 5 minutes under 25 degree-C water bath after 2.85 ml addition, 50microl addition of the copper chloride solution (0.2% CuCl2 and H2O) is carried out, it is stirred, and colorimetry is carried out by 560nm. For the contrast, the purified water was added instead of the sample, and the blank was measured only with 0.2M ammonium-sulfate solution except a sample and XOD liquid. SOD activity defined the time of absorbance change being checked 50% as one unit (U), and computed it by the following formulas.

SOD =(U/ml) [(contrast OD-** ** rank OD)/(sample OD-** ** rank OD)-1] x20[0025] The result is shown in Table 2.

[Table 2]

表2 菌体外ＳＯＤ様比活性 (U／ｍｇﾀﾝﾊﾟｸ)経時変化に及ぼす金属イオンの影響

添加金属*1	培養（時間） 6	19	26
無添加（対照）	11.2±4.1*2	16.2±1.5	37.8±4.8
酸化亜鉛	12.8±2.3	*13.2±1.0	36.8±4.6
塩化マンガン	*20.0±2.9	**37.8±3.9	*49.2±3.2
混合物(酸化亜鉛＋塩化マンガン)	*23.6±3.3	25.6±7.8	34.5±1.0

*1:Davis培地に酵母エキス (0.1%)と炭酸カルシウム (1%)を加えたものを基礎培地(pH7.0)とした。金属濃度:0.1mM
*2:平均値±標準偏差 (n=3)
*: P < 0.05 **: P < 0.01 U: 活性単位

[0026] As shown in Table 2, when it mixed and added, independent or the result increase of remarkable SOD specific activity outside a biomass is accepted to be in the early stages of incubation was obtained for the zinc oxide and the manganese chloride.

[0027] example 1 the result more than skin stimulative test (safety): -- a basis -- the stimulative test of the charge of makeup

which blended ******, manganese, and zinc was performed Synizesis **** of the following (Table 3) solutions was carried out on the tape as an example of combination of the charge of makeup at the filter paper on the forearm inside section skin of 20microl ********** and an adult boy (n= 1), it observed about the skin stimulative of 24 hours after, and the result shown in Table 4 was obtained.

[0028]

[Table 3]

表3 化粧料配合例

	サンプル番号		
配合例	1	2	3（対照）
マンガン（100μM）	+	−	−
亜鉛（100μM）	+	−	−
$CaCO_3$(1%)	−	+	−
グリセリン（5%）	+	+	+
精製水（計10ml）			

[0029] In order that sample numbers 1 and 2 might investigate about the compounding agent of manganese and zinc, and stimulative [of a calcium carbonate], the sample number 3 was created as a contrast. Stimulative [of a Red Spot / observation and stimulative] do not accept the judgment by the naked eye at all, but it was satisfactory at safety in any way.

[0030]

[Table 4]

表4 皮膚刺激試験成績（24時間貼布）

サンプル番号	除去30分後	除去24時間後	除去48時間後
1	−	−	−
2	−	−	−
3（対照）	−	−	−

[0031] Next, the example of prescription of such a charge of makeup is illustrated to this invention. Weight % shows all blending ratio of coals. In addition, this invention cannot be overemphasized by not being limited at all to the following examples of prescription by such charge of makeup.

[0032] Example 2 Composition of face toilet [Table 5]

組成	重量（%）
塩化マンガン	0.002
酸化亜鉛	0.0008
グリセリン	5.0
エタノール	8.0
ポリオキシエチレンソルビタンモノラウレート	1.3
香料	適量
防腐剤	適量
色素	適量
精製水	残量

[0033] Example 3 Composition of a calamine lotion [Table 6]

組成	重量（%）
エタノール	14.0
グリセリン	4.0
酸化亜鉛	0.0008
塩化マンガン	0.002
炭酸カルシウム	0.1
ベンガラ	0.15
カオリン	1.0
カンファー	0.15
香料	適量
精製水	残量

[0034] Example 4 Composition of a skin lotion [Table 7]

組成	重量（%）
塩化マンガン	0.002
酸化亜鉛	0.0008
ジプロピレングリコール	1.0
精製水	残量

[0035] Example 5 Composition of skin cream [Table 8]

組成		重量（%）
油相	ミリスチン酸	30.0
	オクチルドデシル	
	エタノール	6.0
	セチルパルミテート	3.0
	セスギステアリン酸ソルビタン	4.0
水相	塩化マンガン	0.002
	酸化亜鉛	0.0008
	グリセリン	5.0
	精製水	残量

[0036]

[Effect of the invention] By using the charge of makeup which carries out optimum-dose inclusion of the zinc and manganese of

a metal ion by this invention, healthy training of a skin useful resident bacterial flora and SOD Mr. active-substance secretion from a biomass are promoted, and effects, such as growth prevention of a skin detrimental bacillus, active oxygen trauma prevention of a skin cell, and skin cellular-senescence prevention, are expected.

[Translation done.]